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Imidazolinone-Resistant Wheat Acetolactate Synthase In Vivo Response to Imazamox[1]

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Abstract: Several experiments were conducted to evaluate the utility of an in vivo acetolactate synthase (ALS) assay for comparing sensitivity to imazamox among imidazolinone-resistant wheat cultivars/lines. Ten single-gene imidazolinone-resistant winter wheat cultivars/lines, one two-gene and four single-gene imidazolinone-resistant spring wheat cultivars/lines, and three pairs of heterozygous and homozygous imidazolinone-resistant winter wheat lines were evaluated in the assay experiments. Additionally, a dose-response assay was conducted to evaluate the tolerance of several imidazolinone-resistant wheat cultivars to imazamox on a whole plant level. The I_{50} value (i.e., the imazamox dose that inhibited ALS activity by 50%) of the winter wheat cultivar 'Above' was 54 to 84% higher than the I_{50} values of 99-420, 99-433, and CV-9804. However, based on the results of this study, it is unclear whether genetic background or market class (hard red winter vs. soft white winter) influences the level of ALS inhibition by imazamox. Teal 15A, the two-gene imidazolinone-resistant spring wheat cultivar, had an I_{50} value that was two to three times greater than the I_{50} value of the single-gene imidazolinone-resistant spring wheat cultivars/lines. The heterozygous imidazolinone-resistant wheat lines had I_{50} values that were 69 to 81% less than the I_{50} values of the homozygous lines. In the whole plant dose response, the R_{50} values (i.e., the imazamox dose that reduced biomass by 50%) of the susceptible cultivars Brundage 96 and Conan were 15 to 17 times less than the homozygous single-gene imidazolinone-resistant winter and spring cultivars/lines, whose R_{50} values were about 1.7 times less than the R_{50} value of the two-gene imidazolinone-resistant spring wheat line, Teal 15A. The results of the in vivo ALS imazamox assays and the whole plant imazamox dose-response assay were similar, indicating that the in vivo assay can be used to accurately and quickly compare resistance between imidazolinone-resistant wheat cultivars/lines.

Nomenclature: Imazamox, wheat, *Triticum aestivum* L.

Additional index words: Crop safety, herbicide tolerance, herbicide-resistant wheat, in vivo ALS assay.

Abbreviations: ALS, acetolactate synthase; CPCA, 1,1-cyclopropanedicarboxylic acid; I_{50}, imazamox dose that inhibited ALS activity by 50%; KARI, keto-acid reductoisomerase; R_{50}, imazamox dose that reduced biomass 50%.

INTRODUCTION

Imidazolinone herbicides are widely used because they have a broad spectrum of weed control activity, low usage rates, and low mammalian toxicities (Shaner et al. 1996). These herbicides inhibit acetolactate synthase (ALS, also known as acetohydroxyacid synthase, EC 4.1.3.18), the first enzyme unique to the biosynthesis of the branched-chain amino acids valine, leucine, and isoleucine (Shaner et al. 1984; Singh et al. 1988). The development of wheat cultivars with imidazolinone-insensitive ALS makes it possible to control weeds in wheat with previously nonselective imidazolinone herbicides (Newhouse et al. 1992). Applying the herbicide imazamox to imidazolinone-resistant wheat provides an unprecedented opportunity to selectively control closely related grass weeds such as jointed goatgrass (*Aegilops cylindrica* Host #3) (Ball et al. 1999). However, there are reports of imazamox injuring imidazolinone-resistant spring and winter wheat (Ball et al. 1999; Rauch and Thill 2002). In several Pacific Northwest field trials, tolerance to imazamox has varied between imidazolinone-resistant wheat cultivars, years, and locations (T. Rauch,

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personal communication). Sensitivity to chlorsulfuron, another ALS-inhibiting herbicide used for weed control in wheat, varied among three wheat cultivars (Dastgheib and Field 1998). Consequently, imidazolinone-resistant wheat breeders are continually selecting for cultivars that exhibit high levels of resistance.

Imidazolinone-resistant wheat was developed through seed mutagenesis of 'Fidel' winter wheat and selection with the herbicide imazethapyr (Newhouse et al. 1992). The resulting four wheat plants, named FS1 (i.e., Fidel selection 1), FS2, FS3, and FS4, had a single semidominant gene coding for imidazolinone-resistant ALS (Newhouse et al. 1992) located on the D genome (Seefeldt et al. 1998). Allopolyploids commonly have multiple genes for ALS production (Rutledge et al. 1991; Swanson et al. 1989), and each gene is derived from a different progenitor species (Mazur et al. 1987). Wheat, an allohexaploid (2N = 42) with genetic material from diploid genomes A, B, and D (Kimber and Sears 1987), is believed to have at least one ALS production gene from each genome. It is estimated that each gene coding for ALS produces approximately one-third of the overall ALS in a wheat plant (M. Dahmer, personal communication). Most current imidazolinone-resistant winter wheat cultivars/lines have a single gene coding for resistant ALS, because resistance was introduced through backcrosses with one of these initial four resistant selections. Unacceptable crop injury with imazamox in single-gene imidazolinone-resistant spring wheat has led to the development of spring wheat cultivars with two imidazolinone-resistant ALS genes (M. Dahmer, personal communication).

When developing imidazolinone-resistant wheat cultivars, breeders make selections on the basis of sensitivity to imazamox. A common screening method involves treating two- to four-leaf wheat plants with a high dose of imazamox and selecting the plants that survive. Sometimes both heterozygous and homozygous imidazolinone-resistant plants survive the imazamox treatment, and the level of imidazolinone resistance cannot be visibly distinguished. These plants must be grown until maturity, at which point their seed is harvested, planted, and the subsequent seedlings are treated with imazamox. The segregation ratios are then evaluated to determine whether the parent plant was heterozygous or homozygous for the imidazolinone resistance trait. This procedure is slow because of the time period between generations and the time required for susceptible plants to die from an imazamox treatment. Additionally, this screening method does not provide a reliable quantitative means for comparing imazamox resistance between cultivars.

Gerwick et al. (1993) developed a rapid in vivo ALS assay to distinguish between ALS-inhibiting herbicide-resistant and herbicide-susceptible plants. The assay uses 1,1-cyclopropanedicarboxylic acid (CPCA) to inhibit keto-acid reductoisomerase, EC 1.1.1.86 (KARI), the enzyme immediately following production of acetolactate in the branched-chain amino acid biosynthetic pathway. Inhibition of KARI results in an accumulation of acetolactate, the product of the ALS catalyzed reaction. In the presence of an ALS inhibitor (imazamox), carbon flow from pyruvate is inhibited in susceptible plants and no acetolactate is produced. ALS activity is indirectly measured by converting acetolactate to acetoin, which is then quantified with a Westerfield (1945) colorimetric assay.

The objective of this study was to test an in vivo ALS assay as a selection tool for evaluating imazamox resistance among imidazolinone-resistant wheat cultivars/lines, and to distinguish between homozygous and heterozygous resistant cultivars/lines. Additionally, a whole plant assay was conducted to determine whether whole wheat plant response to imazamox was similar to in vivo inhibition of ALS with imazamox.

MATERIALS AND METHODS

In Vivo ALS Assay Experiments. Experiments were conducted at the University of Idaho in Moscow, ID using seed donated by public and private industry wheat breeders (Tables 1 and 2). Four wheat seeds of each cultivar/line were planted 2 cm deep in 10.5 cm^2 pots filled with potting soil and placed in a growth chamber under 200 $\mu E/m^2/s$ of radiation with a 16-h photoperiod and 20 and 16 C day and night temperatures, respectively. Plants were watered and fertilized daily.

ALS activity was measured when plants were in the four-leaf stage using a modified version of the in vivo assay procedure described by Gerwick et al. (1993) and Simpson et al. (1995). A stock incubation solution containing 500 μM/L of CPCA, 10% v/v of Murashige and Skoog basal salts, 10 mM/L KH_2PO_4 buffer (pH 6.0), and 0.5% w/v alanine was prepared immediately before each assay. Imazamox was added to a subsample of the incubation solution to obtain a concentration of 25 μM/L. Assays were conducted in 96-well microtiter plates and 100 μl of the incubation solution, with or without imazamox, was pipetted into each well. Serial dilutions were performed using the stock solution to achieve doses of 0.78, 1.56, 3.13, 6.25, 12.5, and 25 μM/L imazamox.

Table 1. Market class, acetolactase synthase (ALS) resistance gene, and recurrent parent of imidazolinone-resistant cultivars/lines in the winter wheat and spring wheat in vivo ALS imazamox dose-response experiments and the whole plant imazamox dose-response experiment.

Cultivar/line	Market class[a]	ALS resistance and location[b]	Recurrent parent[c]
AP 602 CL	HRS	Single gene, B	Gunner
Exp 205 CL	HRS	Single gene, B	Gunner
Triangle	HRS	Single gene, D	WestBred 926
Teal 15A	HRS	Double gene	CDC Teal
CA06BR	HRS	Single gene, D	Conan
BZ9M99 1019	HRS	Single gene, D	WestBred 926
WestBred 926	HRS	Susceptible	WestBred 926
Conan	HRS	Susceptible	Conan
AP 502 CL	HRW	Single gene, D	TAM-110
TAM-110 CL	HRW	Single gene, D	TAM-110
Above	HRW	Single gene, D	TAM-110
CO980879	HRW	Single gene, D	TAM-110
99-433	SWW	Single gene, D	Brundage 96
99-437	SWW	Single gene, D	Lambert
IDO 587	SWW	Single gene, D	Stephens
99-420	SWW	Single gene, D	87-52814A (814A)
ORCF-101	SWW	Single gene, D	Malcom/Stephens/Madsen
CV-9804 (FS4)	SRW	Single gene, D	Fidel
TAM-110	HRW	Susceptible	TAM-110
Brundage 96	SWW	Susceptible	Brundage 96

[a] Abbreviations: HRS, hard red spring wheat; HRW, hard red winter wheat; SWW, soft white winter wheat; SRW, soft red winter wheat.

[b] Single gene, B and D, denote a single imidazolinone resistance gene located on the B and D genomes, respectively. Double gene is apparent two-gene imidazolinone resistance with uncharacterized gene locations. Susceptible is nonimidazolinone resistant.

[c] Indicates the susceptible parent into which the imidazolinone-resistant trait was incorporated.

Each imazamox dose well was paired with a nontreated control well containing the incubation solution without imazamox.

The main shoots of the wheat plants were removed by clipping 3 cm above the soil surface. Using a razor blade, two 5-mm segments were cut from the primordial leaf portion of each shoot and randomly placed in a control well or the corresponding imazamox dose well. Plates were incubated for 24 h under a fluorescent light source at 150 $\mu E/m^2/s$, placed in a freezer at -80 C for about 30 min until solutions were frozen, and then thawed for 15 min in an oven at 60 C. Acetolactate was decarboxylated to acetoin by adding 25 μl of 5% H_2SO_4 to each well and heating the plates for 20 min in an oven at 60 C. A solution was prepared containing 0.25 and 2.5% (w/v) of creatine and α-napthol, respectively, in 2 N NaOH, and 150 μl was pipetted into each well. Plates were placed in a 60 C oven for 15 min to facilitate color change, and absorbance was measured spectrophotometrically at 532 nm using a plate reader.[3]

One experiment included 2 susceptible winter wheat cultivars and 10 single-gene homozygous imidazolinone-resistant winter wheat cultivars/lines (Table 1). Imidazolinone-susceptible lines were included to ensure that positive color responses were due to the presence of ALS rather than to bacterial contamination. Another experiment compared ALS activity among five single-gene ho-

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Table 2. Parental information, resistance trait expression, and recurrent parent of winter wheat cultivars/lines in the heterozygous-homozygous and maternal-paternal parent in the in vivo acetolactase synthase imazamox dose-response experiment.

Line	Parent		Resistance trait expression[a]	Recurrent parent[b]
	Maternal	Paternal		
99-422	99-422	99-422	Homozygous	814A
99-422/814A	99-422	814A	Heterozygous	814A
99-429	99-429	99-429	Homozygous	Brundage 96
Brundage 96/99-429	Brundage 96	99-429	Heterozygous	Brundage 96
Brundage 96	Brundage 96	Brundage 96	Susceptible	Brundage 96
99-435	99-435	99-435	Homozygous	Lambert
Lambert/99-435	Lambert	99-435	Heterozygous	Lambert
99-435/Lambert	99-435	Lambert	Heterozygous	Lambert

[a] Homozygous or heterozygous single gene imidazolinone resistance.

[b] Indicates the susceptible parent into which the imidazolinone-resistant trait was incorporated.

mozygous imidazolinone-resistant hard red spring wheat cultivars/lines, one two-gene homozygous imidazolinone-resistant hard red spring cultivar/line, and two susceptible hard red spring cultivars. A third experiment was conducted to determine whether the in vivo assay could be used to distinguish between heterozygous and homozygous imidazolinone-resistant wheat lines. Three homozygous imidazolinone-resistant winter wheat lines, 99-422, 99-429, and 99-435, were crossed with their respective susceptible parent lines to produce 99-422/814A, Brundage 96/99-429, 99-435/Lambert, and Lambert/99-435, which are heterozygous for the imidazolinone resistance trait (Table 2). In each cross, the first line listed represents the maternal parent and the second line is the paternal parent. Lambert/99-435 and 99-435/Lambert are reciprocal lines and were used to evaluate the influence of maternal parent on ALS sensitivity to imazamox. The study also included Brundage 96 as an imidazolinone-susceptible control. Homozygosity was confirmed by progeny testing for herbicide resistance. Lines that were 100% imidazolinone resistant in the progeny test were used as parents to produce the heterozygous lines and as the seed source for the homozygous lines used in this study.

Because of the time required to collect stem segments and the influence of incubation time on color development, all assays were conducted using a randomized complete block design and blocked by replication. The winter and spring wheat experiments had four replications, whereas the amount of seed available limited the homozygous-heterozygous and maternal-paternal parent experiments to three replications. All experiments were repeated once. Absorbance data were analyzed as a percentage of absorbance in the corresponding nontreated control well. Percentage data were evaluated and did not require transformation to meet the assumptions of the statistical analyses used. The relationship between imazamox dose and ALS activity was described using the following dose response model:

$$y = a(\exp[-b(x^c)]) + e \qquad [1]$$

where y is the estimated ALS activity (percent of the nontreated control) as a function of imazamox dose (x); a is the intercept; b and c are parameters that control the steepness and shallowness of the curve, respectively; and e is an error term under the usual assumptions of regression analysis (i.e., $e \sim N(0, \sigma^2)$).

This model describes a decreasing response that gradually approaches zero with increased dose and is an extension of the typical exponential model commonly used in dose-response problems (Ratowsky 1990). While differing somewhat from the S-shaped log-logistic model reported by Seefeldt et al. (1995), this exponential form was found to supply the necessary flexibility required in this case, which the log-logistic model could not provide. I_{50} values (i.e., the dose at which there is a 50% response level), can be computed from Equation 1 as:

$$I_{50} = ([\ln(50) - \ln(a)]/[-b])^{**}(1/c) \qquad [2]$$

where a, b, and c have the same definitions as in Equation 1. Comparisons of I_{50} values were carried out using single degree of freedom contrasts and 95% confidence intervals based on a full dummy variable model that simultaneously estimated all cultivars. Data analysis revealed a slight treatment by repetition interaction, however, it was nonsevere, and treatments were essentially ordered the same way in both experiments, thus data are presented as an average. When appropriate, contrasts of I_{50} values were performed to make specific comparisons. Estimation was carried out with nonlinear regression analysis using PROC NLMIXED (Figure 1) assuming a normal distribution with a mean given by Equation 1, and common variance, sigma (SAS 1999). As the name of the procedure implies, PROC NLMIXED can be used to estimate nonlinear mixed models, however, it can also estimate models in the more familiar nonlinear fixed case, as was performed here. This procedure is preferred because (1) it allows for flexibility in the response distribution, (2) it uses a robust maximum likelihood estimation technique for estimation, and (3) it allows for the computation and statistical comparison of quantities such as the I_{50} value given in Equation 2.

Whole Plant ALS Dose-Response Assay. A whole plant dose-response assay was conducted to determine whether whole plant response to imazamox was similar to in vivo inhibition of ALS with imazamox. Only five cultivars/lines were used for the whole plant assay because seed stocks were inadequate for other cultivars/lines. Individual seeds of Teal 15A, CA06BR, Conan, CV-9804, and Brundage 96 were planted 2 cm deep in a 10.5 cm^2 pots filled with potting soil. Market classes, resistance types, and recurrent parents are listed in Table 1. Plants were grown in a greenhouse under natural and supplemental light with a 14-h photoperiod, watered daily, and fertilized every 7 d. Plants at the four-leaf stage were sprayed with imazamox at 1.4, 2.8, 5.6, 11.2, 22.4, 44.8 (registered use rate), 89.6, 179.2, 358.4, 716.8, and 1,433.6 g/ha with a greenhouse cabinet sprayer calibrated to deliver 150 L/ha at 280 kPa. A nontreated control was included. Plants were harvested 14 d after treatment

by cutting the shoots at the soil surface, placing them in paper envelopes, and drying at 60 C for 48 h. Shoot dry weights were recorded and data were analyzed as a percentage of the biomass in the nontreated control. The experimental design was a randomized complete block with four replications and was repeated once. Data were subjected to nonlinear regression analysis using Equation 1, where *y* is the estimated biomass (percent of the control) as a function of imazamox dose (*x*), *a* is the amount of biomass produced in the absence of imazamox (intercept), and *b* and *c* control the shape of the curve. R_{50} values were determined by calculating the imazamox dose at which biomass was reduced by 50% using a modified form of Equation 2. Cultivar/line comparisons were made using R_{50} values and upper and lower 95% confidence intervals.

RESULTS AND DISCUSSION

Winter Wheat ALS Assays. The lowest imazamox dose in the assay, 0.78 μM/L, inhibited ALS activity of the susceptible cultivars, Brundage 96 and TAM 110, 90% or more (Rainbolt 2003). Consequently, the curves for these two cultivars were too steep to fit the model and are not presented.

For the imidazolinone-resistant cultivars all model parameters (Table 3) were significant ($P < 0.0001$) and the resulting residuals were adequate in trend, magnitude, and distribution. Estimated curves and fit for all cultivars/lines were similar to those of TAM-110 CL (Figure 2). Estimated curves for all cultivars/lines are presented in Figure 3. Due to variation in absorbance data between paired wells, data for the nontreated control often exceeded 100%. Thus, the estimated intercept (parameter a) for some cultivars/lines is greater than 100%. Calculated I_{50} values ranged from 1.4 (99-433) to 2.6 μM/L (Above) imazamox (Figure 4). The I_{50} value of Above was 54 to 83% higher than the I_{50} values of 99-420, 99-433, and CV-9804. The I_{50} of 99-433 was also 42 to 47% less than the I_{50} values of TAM-110 CL and CO980879. The four hard red winter wheat cultivars/lines, which all had a TAM-110 genetic background (Table 1), had similar I_{50} values ranging from 2.0 to 2.6 μM/L imazamox. Likewise, the I_{50} values for the soft white winter cultivars/lines were similar, and ranged from 1.4 to 2.1 μM/L imazamox, despite having different recurring parents (Table 1), indicating that genetic background may not influence the sensitivity of ALS to imazamox. A contrast of I_{50} values between market classes revealed that the hard red cultivars/lines had a higher average I_{50} value ($P = 0.0012$). Although statistically significant, it is unclear whether this small level of difference is biologically meaningful. Furthermore, the difference may be a result of Above, the cultivar with the highest I_{50} value (2.7 μM/L imazamox), being in the hard red class, whereas 99-433, the line with the lowest I_{50} value (1.4 μM/L imazamox), is in the soft white class. The I_{50} values of the other hard red and soft white winter cultivars ranged from only 1.7 to 2.1 μM/L imazamox. Thus, based on the results of this study it is unclear whether genetic background or market class plays a role in the sensitivity of ALS to imazamox. The I_{50} values of all winter wheat cultivars/lines tested, except Above, were not different than the I_{50} value of CV-9804, which is a line developed through seed increase of FS4. Thus, it appears that the ALS sensitivities of most cultivars/lines to imazamox are similar to those of FS4, one of the original four imidazolinone-resistant selections.

Response of ALS to imazamox was different among the winter wheat cultivars/lines tested in the in vivo as-

A

```
proc nlmixed data=wwals;
      parms a = 100 b=.5 c=.5 sigma = 50;
      *bounds c<=2;
      mu = a*exp(-b*(trmt2**c));
      model perc ~ normal(mu, sigma);
      predict mu out = predict;
      predict perc - mu out = resid;
      estimate 'ED50' ((log(50) - log(a))/(-b))**(1/c);
      predict ((log(50) - log(a))/(-b))**(1/c) out = ed50;
      by cult;
run;
```

B

```
proc nlmixed data=wwals;
      parms a1 = 120.2 b1=.68 c1=.48
            a2 = 96.7 b2=.35 c2=.65
            a3 = 109.7 b3=.57 c3=.45
            a4 = 120.3 b4=.77 c4=.39
            a5 = 103.2 b5=.51 c5=.51
            a6 = 116.3 b6=.69 c6=.41
            a7 = 110.2 b7=.59 c7=.44
            a8 = 112.4 b8=.59 c8=.43
            a9 = 110.2 b9=.56 c9=.48
            a10 = 108.9 b10=.55 c10=.47
            sigma = 50;

            if cult='814CL' then mu = a1*exp(-b1*(trmt2**c1));
            else if cult= 'ABOVE' then mu = a2*exp(-b2*(trmt2**c2));
            else if cult= 'AP5CL' then mu = a3*exp(-b3*(trmt2**c3));
            else if cult= 'BRUCL' then mu = a4*exp(-b4*(trmt2**c4));
            else if cult= 'CO980' then mu = a5*exp(-b5*(trmt2**c5));
            else if cult= 'FIDEL' then mu = a6*exp(-b6*(trmt2**c6));
            else if cult= 'ID587' then mu = a7*exp(-b7*(trmt2**c7));
            else if cult= 'LAMCL' then mu = a8*exp(-b8*(trmt2**c8));
            else if cult= 'ORCF1' then mu = a9*exp(-b9*(trmt2**c9));
            else if cult= 'TAMCL' then mu = a10*exp(-b10*(trmt2**c10));

      model perc ~ normal(mu, sigma);

      ED501=((log(50) - log(a1))/(-b1))**(1/c1);
      ED502=((log(50) - log(a2))/(-b2))**(1/c2);
      ED503=((log(50) - log(a3))/(-b3))**(1/c3);
      ED504=((log(50) - log(a4))/(-b4))**(1/c4);
      ED505=((log(50) - log(a5))/(-b5))**(1/c5);
      ED506=((log(50) - log(a6))/(-b6))**(1/c6);
      ED507=((log(50) - log(a7))/(-b7))**(1/c7);
      ED508=((log(50) - log(a8))/(-b8))**(1/c8);
      ED509=((log(50) - log(a9))/(-b9))**(1/c9);
      ED5010=((log(50) - log(a10))/(-b10))**(1/c10);

      estimate 'test' ((ED502+ED503+ED505+ED5010)*0.25)-
                  ((ED501+ED504+ED507+ED508+ED509)*.20);

run;
```

Figure 1. Example of SAS programming code using PROC NLMIXED for (A) the maximum likelihood dose-response model and I_{50} calculations and (B) single degree of freedom full dummy variable contrast.

Table 3. Model parameter estimates and standard errors for winter wheat cultivars/lines in the in vivo acetolactase synthase imazamox dose-response experiment.

Cultivar/line	Parameter[a]	Estimate	Standard error
AP 502 CL	a	109.75	4.39
	b	0.57	0.06
	c	0.45	0.04
TAM-110 CL	a	108.89	3.63
	b	0.55	0.05
	c	0.47	0.04
Above	a	96.78	4.40
	b	0.35	0.03
	c	0.65	0.04
ORCF-101	a	110.19	4.36
	b	0.56	0.06
	c	0.48	0.05
99-433	a	120.34	5.79
	b	0.77	0.07
	c	0.39	0.04
99-437	a	112.36	5.33
	b	0.59	0.07
	c	0.43	0.05
IDO 587	a	110.18	3.97
	b	0.59	0.05
	c	0.44	0.04
99-420	a	120.22	5.93
	b	0.68	0.07
	c	0.48	0.06
CO980879	a	103.15	2.89
	b	0.51	0.04
	c	0.51	0.03
FS4	a	116.34	5.17
	b	0.69	0.06
	c	0.41	0.04

[a] Abbreviations: a, intercept; b, steepness of the curve; c, shallowness of the curve.

say, indicating that the assay can be used to successfully evaluate resistance to imazamox. However, in field trials, the response of a single imidazolinone-resistant cultivar to imazamox can vary between years and locations. Thus, it is unclear whether imazamox injury observed in field situations is a result of inherent differences in ALS activity, other biological and environmental factors, or a combination of these. One possible explanation for varying levels of resistance to imazamox is that different levels of seedling vigor and growth rate among cultivars results in different rates of imazamox metabolism. Highly vigorous cultivars, such as Above, are likely to be more tolerant to imazamox than slower growing cultivars.

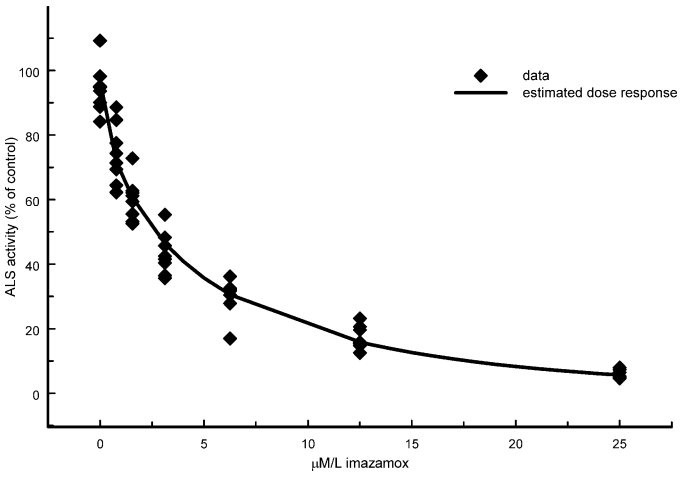

Figure 2. Estimated dose-response curve and actual data for Above, a single-gene homozygous resistant imidazolinone-resistant winter wheat cultivar in the in vivo ALS imazamox dose-response experiment.

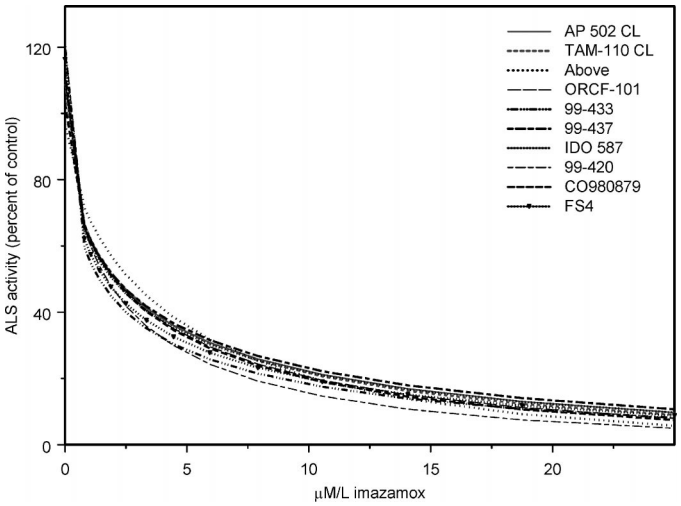

Figure 3. Estimated dose-response curves for imidazolinone-resistant winter wheat cultivars/lines in the in vivo ALS imazamox dose-response experiment.

Spring Wheat ALS Assays. ALS activity of the susceptible spring wheat cultivars Conan and Westbred 926 was inhibited by 85 and 95%, respectively, by 0.78 µM/

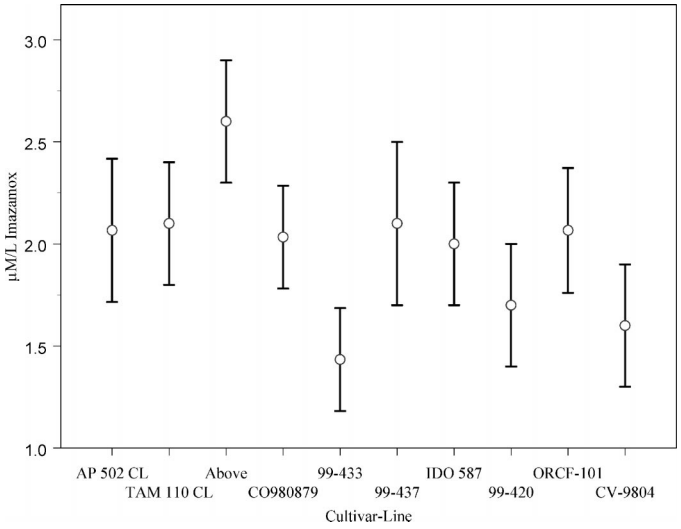

Figure 4. Calculated I_{50} values and upper and lower 95% confidence intervals for imidazolinone-resistant winter wheat cultivars/lines in the in vivo ALS imazamox dose-response experiment.

Table 4. Model parameter estimates and standard errors for spring wheat cultivars/lines in the in vivo acetolactase synthase imazamox dose-response experiment.

Cultivar/line	Parameter[a]	Estimate	Standard error
AP 602 CL	a	122.66	4.77
	b	0.71	0.06
	c	0.46	0.04
Exp 205 CL	a	121.20	5.72
	b	0.71	0.07
	c	0.38	0.04
Triangle	a	116.68	5.58
	b	0.67	0.07
	c	0.43	0.05
Teal 15A	a	107.81	3.11
	b	0.31	0.03
	c	0.55	0.04
CA06BR	a	104.57	3.35
	b	0.47	0.04
	c	0.54	0.04
BZ9M99-1019	a	122.85	6.67
	b	0.76	0.07
	c	0.36	0.04

[a] Abbreviations: a, intercept; b, steepness of the curve; c, shallowness of the curve.

L imazamox (Rainbolt 2003). The data for these two cultivars do not fit the model.

For the imidazolinone-resistant cultivars, all model parameters (Table 4) were significant ($P < 0.0001$), and the resulting residuals were adequate in trend, magnitude, and distribution. Estimated curves are presented in Figure 5. Teal 15A, the two-gene imidazolinone-resistant line, had a higher I_{50} value (5.2 μM/L imazamox) than the single-gene imidazolinone-resistant cultivars/lines (1.6 to 2.3 μM/L imazamox) ($P < 0.0001$) (Figure 6). I_{50} values were not different among the single-gene imidazolinone-resistant cultivars/lines. Recurrent parent and resistance gene location (B genome compared to D genome) (Table 1) did not affect ALS sensitivity to imazamox in the single-gene spring wheat cultivars/lines tested. Based on the limited results of this experiment, the assay can be used to identify one- and two-gene homozygous imidazolinone-resistant spring wheat cultivars/lines.

The two-gene imidazolinone-resistant cultivar/line, Teal 15A, was developed because crop injury within the single-gene imidazolinone-resistant spring wheat cultivars/lines was unacceptable. It is unknown why crop injury occurred more frequently in the single-gene imidazolinone-resistant spring cultivars than within the single-gene winter cultivars (personal communication, Mark Dahmer). Although the spring and winter wheat imidazolinone-resistant cultivars were tested in separate experiments and cannot be compared statistically, the range of I_{50} values from the in vivo ALS assays were similar (1.6 to 2.3 and 1.4 to 2.6 μM/L imazamox for single-gene spring and winter wheat cultivars/lines, respectively).

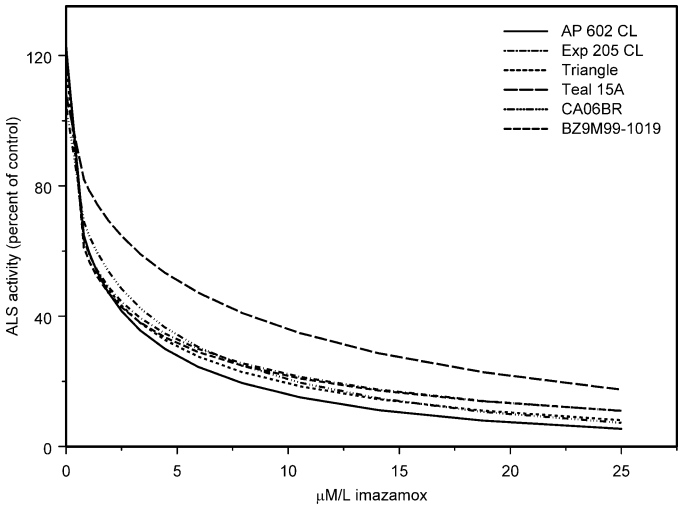

Figure 5. Estimated dose-response curves for imidazolinone-resistant spring wheat cultivars/lines in the in vivo ALS imazamox dose-response experiment.

Homozygous-Heterozygous and Maternal-Paternal Parent Assays. Data for Brundage 96, the susceptible cultivar, did not fit the model (Rainbolt 2003). P values were < 0.0001 for all model parameters (Table 5), and predicted curves are presented in Figure 7. The homozygous resistant lines included in the experiment were not different from each other and had I_{50} values of 1.5 to 2.0 μM/L imazamox (Figure 8). The heterozygous resistant lines were also not different from each other and had I_{50} values ranging from 0.38 to 0.52 μM/L imazamox. However, the average I_{50} values of the homo-

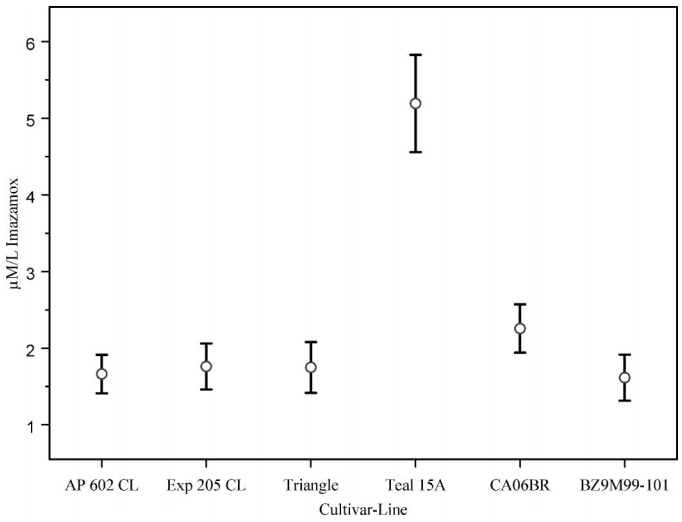

Figure 6. Calculated I_{50} values and upper and lower 95% confidence intervals for imidazolinone-resistant spring wheat cultivars/lines in the in vivo ALS imazamox dose-response experiment.

Table 5. Model parameter estimates and standard errors for homozygous and heterozygous imidazolinone-resistant cultivars lines in the in vivo acetolactase synthase dose-response experiment.

Cultivar/line	Parameter[a]	Estimate	Standard error
99-422	a	104.91	3.15
	b	0.57	0.04
	c	0.50	0.04
99-422/814A	a	130.99	6.26
	b	1.20	0.07
	c	0.34	0.03
99-429	a	121.92	4.09
	b	0.75	0.05
	c	0.41	0.03
Brundage 96/99-429	a	132.71	12.52
	b	1.35	0.13
	c	0.34	0.05
99-435	a	111.59	4.45
	b	0.59	0.06
	c	0.44	0.04
Lambert/99-435	a	140.61	10.94
	b	1.34	0.10
	c	0.33	0.04
99-435/Lambert	a	148.69	13.26
	b	1.38	0.11
	c	0.30	0.04

[a] Abbreviations: a, intercept; b, steepness of the curve; c, shallowness of the curve.

zygous and heterozygous resistant lines were different ($P = 0.00273$), indicating that the assay can be used to separate homozygous from heterozygous imidazolinone-resistant lines.

The ability to distinguish between plants that are heterozygous and homozygous for the imidazolinone resistance trait can save plant breeders considerable time when developing imidazolinone-resistant wheat lines. Although the trait for imidazolinone resistance is semidominant, it is not always possible to distinguish between heterozygous and homozygous plants based on whole plant response to an imazamox treatment (J. Hansen, personal communication). Typically, when heterozygous imidazolinone-resistant plants are sprayed with a high rate of imazamox the growing point of the plant is damaged, resulting in a stunted growth form with excessive tilling. Homozygous resistant plants tend to maintain nearly normal growth following treatment with imazamox. Occasionally, there are plants that do not fit clearly into either category (J. Hansen, personal communication).

When developing lines with multiple genes for imidazolinone resistance, plant breeders often incorporate a second resistance gene into a line that is already homozygous for the first resistance gene. The resulting progeny are homozygous resistant for the first gene and heterozygous resistant for the second gene. Breeders allow these progeny to self-pollinate, which results in a population containing plants that are homozygous resistant for both genes, plants that are homozygous resistant for the first gene and heterozygous resistant for the second gene, and plants that are homozygous resistant for the first gene and homozygous susceptible for the second gene. Therefore, identification of these plants based on their whole plant response to imazamox may be extremely difficult.

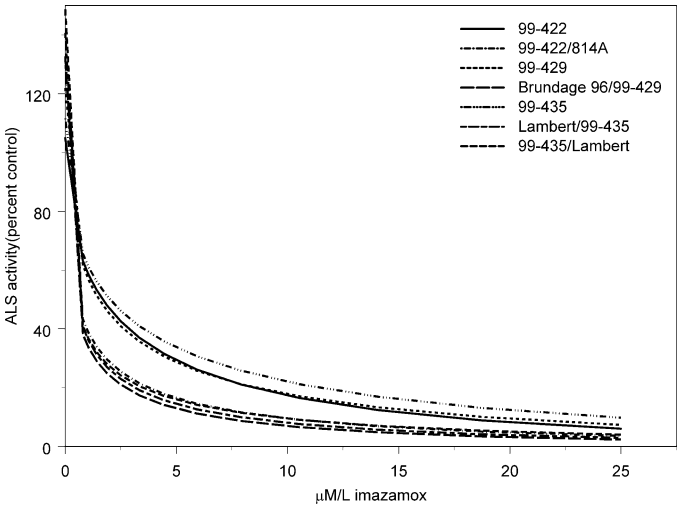

Figure 7. Estimated dose-response curves for imidazolinone-resistant heterozygous and homozygous winter wheat lines in the in vivo ALS imazamox dose-response experiment.

The in vivo assay provides a quantitative measurement for evaluating tolerance to imazamox. However, multiple herbicide rates and replications are necessary for maximum accuracy, which requires multiple plants and makes the method impractical for screening segregating populations. Using a single imazamox rate with this in vivo

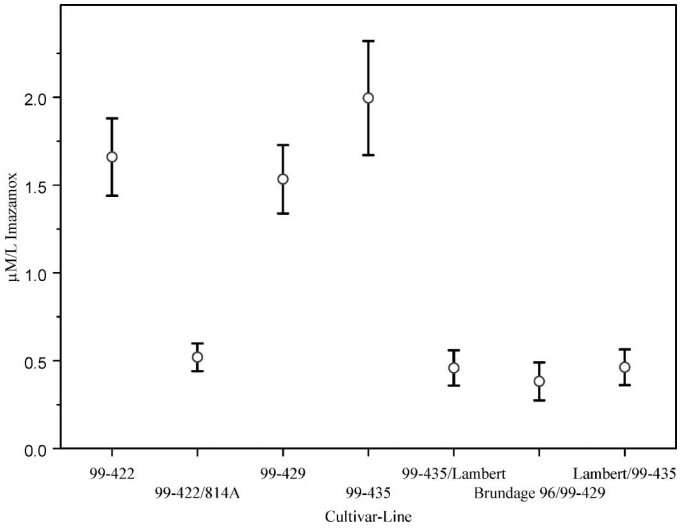

Figure 8. Calculated I_{50} values and upper and lower 95% confidence intervals for imidazolinone-resistant heterozygous and homozygous winter wheat cultivars/lines in the in vivo ALS imazamox dose-response experiment.

Table 6. Model parameter estimates and standard errors for cultivars/lines in the whole plant imazamox dose-response experiment.

Cultivar/line	Parameter[a]	Estimate	Standard error
Teal 15A	a	104.10	1.93
	b	0.02	0.01
	c	1.14	0.16
CA06BR	a	103.57	2.32
	b	0.06	0.02
	c	1.00	0.13
Conan	a	119.95	8.08
	b	0.95	0.08
	c	0.59	0.13
CV-9804	a	108.68	2.37
	b	0.09	0.02
	c	0.82	0.09
Brundage 96	a	139.90	16.78
	b	1.09	0.13
	c	0.39	0.09

[a] Abbreviations: a, intercept; b, steepness of the curve; c, shallowness of the curve.

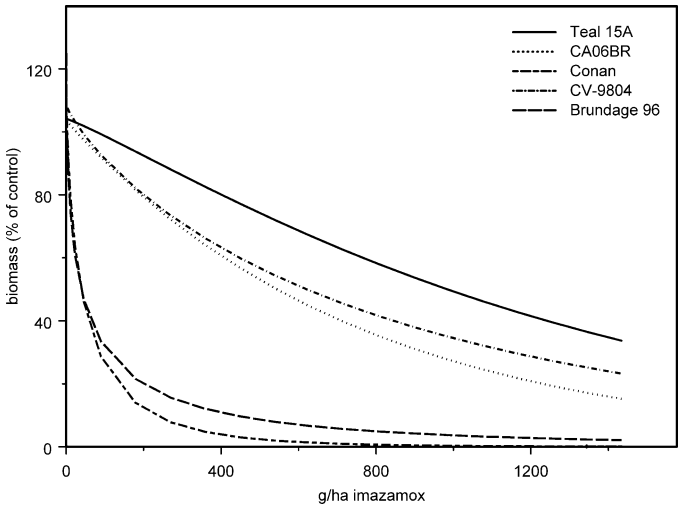

Figure 9. Estimated dose-response curves for wheat cultivars/lines in the whole plant imazamox dose-response assay experiment.

assay technique would allow breeders to screen the individual plants of a segregating population, but due to natural variation between plants it might not be accurate enough to avoid errors.

The I_{50} values for the reciprocal crosses, Lambert/99-435 and 99-435/Lambert, were 0.46 and 0.45 μM/L imazamox, respectively, and did not differ ($P = 0.988$). Based on results of these limited findings, it appears that the maternal parent does not influence expression of the imidazolinone resistance trait.

Whole Plant Dose Response. Model parameter P values ranged from < 0.0001 to 0.0472 (Table 6). Biomass production of the susceptible cultivars Brundage 96 and Conan was almost completely inhibited by imazamox (R_{50} values of 36.7 and 38.5 g/ha imazamox, respectively) compared to the imidazolinone-resistant cultivars/lines (Figures 9 and 10). The single-gene imidazolinone-resistant cultivars/lines CA06BR and CV-9804 had R_{50} values of 573 and 623 g/ha imazamox, respectively, and had similar upper and lower 95% confidence intervals. Biomass of Teal 15A, the two-gene imidazolinone-resistant spring wheat line was inhibited least compared to the other cultivars/lines ($R_{50} = 986$ g/ha imazamox).

Unfortunately, insufficient seed stocks limited the cultivars/lines used in the whole plant assay. Including more cultivars/lines in the whole plant study would have provided more information about potential differences between the single-gene imidazolinone-resistant cultivars/lines on a whole plant level. However, the experiment did show that Teal 15A is approximately twice as tolerant to imazamox as the single-gene imidazolinone-resistant cultivars/lines and that the growth of the imidazolinone-susceptible cultivars is severely inhibited by low doses of imazamox.

The results of the in vivo ALS imazamox assays and the whole plant imazamox dose-response assay cannot be compared statistically. However, both ALS activity and biomass production of the two-gene imidazolinone-resistant cultivar/line (Teal 15A) were inhibited less by imazamox than by the single-gene resistant cultivars/lines, which was inhibited less than the susceptible cultivars. Demonstrating that in vivo inhibition of ALS with imazamox is correlated with reduced growth caused by imazamox on a whole plant level. Dastgheib and Field (1998) compared three wheat cultivars and found that in vivo ALS inhibition by chlorsulfuron corresponded with

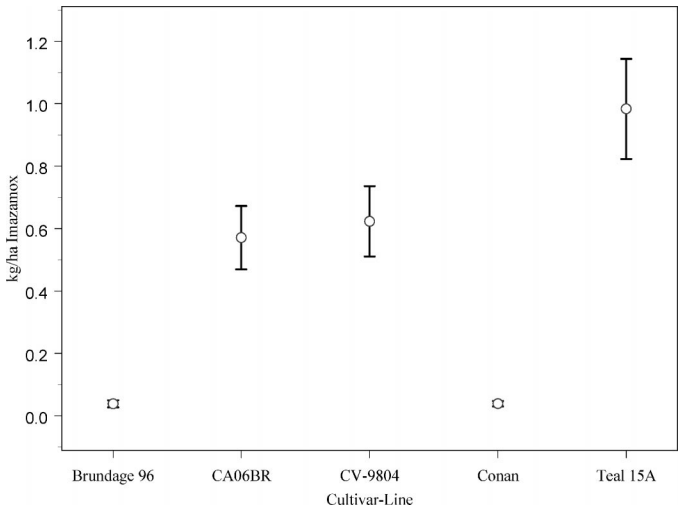

Figure 10. Calculated R_{50} values and upper and lower 95% confidence intervals for cultivars/lines used in the whole plant imazamox dose-response assay experiment.

sensitivity to chlorsulfuron on a whole plant level. Thus, it appears that the in vivo ALS assay is a valid tool for comparing resistance to imazamox between imidazolinone-resistant cultivars/lines. However, because the assay is in vivo rather than in vitro, it is unclear whether the differences observed are due to genetic differences in ALS sensitivity/levels or to other biological factors such as growth rate and vigor.

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